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Research Note

Free Radical Generation During Infection with *Nippostrongylus brasiliensis* (Nematoda) and/or *Eimeria nieschulzi* (Apicomplexa)

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ABSTRACT: To determine if *Eimeria nieschulzi* suppresses *Nippostrongylus brasiliensis*-induced self-cure in Sprague-Dawley rats by inhibiting free radical production, jejunal free radical production was assessed using the Thiobarbituric Acid Assay in rats infected with 1×10^3 third-stage larvae of *N. brasiliensis* and/or 1×10^5 sporulated oocysts of *E. nieschulzi.* Sham infected rats administered saline served as controls. Rats infected with *N. brasiliensis* were killed on day 11 postinoculation (PI) and those infected with *E. nieschulzi* on day 8 PI. Rats infected with both parasites were killed when day 8 PI with *E. nieschulzi* coincided with day 11 PI *N. brasiliensis.* Free radical production was indirectly assessed by quantifying malondialdehyde (MDA), and data were expressed as $\bar{x}$ nM MDA $\times 10^{-2}/\mu g$ mucosal protein. No significant ($P \geq 0.05$) differences in MDA production were observed among the groups. Results of this study show that *E. nieschulzi* does not suppress *N. brasiliensis* self-cure by inhibition of free radical production in Sprague-Dawley rats and, in fact, that this rat strain does not increase jejunal free radical production in response to infection with either parasite.

KEY WORDS: *Nippostrongylus brasiliensis, Eimeria nieschulzi,* free radical production, malondialdehyde, Thiobarbituric Acid Assay.

Smith and Bryant (1989) used a Thiobarbituric Acid (TBA) Assay to show that small intestinal free radical production was associated temporally with the expulsion of *Nippostrongylus brasiliensis* primary infections in female Wistar rats and suggested free radicals play an important role in the self-cure process. These authors focused their studies on the generation and effects of free radicals in rats infected only with *N. brasiliensis.* In nature, however, animals are usually infected with more than 1 species of parasite, and it has been shown that the host reaction to an infection with a single parasite species may be altered in the presence of another species. For example, Bristol et al. (1983) were able to show that Sprague-Dawley rats concurrently infected with *N. brasiliensis* and *Eimeria nieschulzi* had significantly longer helminth patent periods when compared to rats that had only been infected with *N. brasiliensis,* suggesting that the host's immune and inflammatory response to *N. brasiliensis* was suppressed by *E. nieschulzi.* In separate reports, Broaddus et al. (1987) and Upton et al. (1987) demonstrated that *E. nieschulzi* suppressed *N. brasiliensis*-induced intestinal eosinophil lysophospholipase activity and relative peripheral eosinophilia, respectively. Although the mechanism by which *E. nieschulzi* suppresses self-cure of *N. brasiliensis* in Sprague-Dawley rats is unknown, we hypothesized it may be due, in part, to a suppression of free radical production since (1) it has been shown that *E. nieschulzi* suppresses intestinal eosinophilia (Broaddus et al., 1987), and (2) it is known that eosinophils are a major source of the free radicals generated in response to *N. brasiliensis* (Smith and Ovington, 1994). To test our hypothesis, specific pathogen-free mature male Sprague-Dawley *Rattus norvegicus,* each weighing 150–300 g, were used. Each rat was individually housed in an autoclaved cage that contained bedding (wood shavings) and was covered with a wire lid. Food and water were provided *ad libitum.* Rats were infected with 1×10^3 third-stage larvae of *N. brasiliensis* and/or 1×10^5 sporulated oocysts of *E. nieschulzi* and were killed on day 11 of the nematode infection ($N = 5$), day 8 of the coccidian infection ($N = 5$), or when day 8 of the *E. nieschulzi* infection coincided with day 11 of the *N. brasiliensis* infection ($N = 5$). Uninfected sham-treated controls were administered 0.9% saline (NaCl) (w/v) and killed simultaneously along with uninfected-untreated controls. The jejunum was removed, slit open, washed to remove all debris and worms, and the number of worms present quantified. Intestinal mucosa (250 mg) was weighed and homogenized in a Virtis tissue homogenizer in phosphate-buffered saline (PBS),

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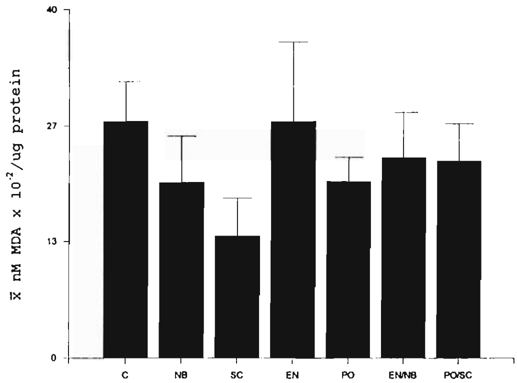

Figure 1. Malondialdehyde (MDA) production (x̄ nM MDA × 10^{-2}/μg protein ± SE) by small intestinal mucosa from rats infected with *Nippostrongylus brasiliensis* (NB) and/or *Eimeria nieschulzi* (EN), from sham-infected control rats that received saline subcutaneously (SC) and/or *per os* (PO), and from uninfected controls (C). N = 5 rats/group. NB = day 11 postinfection (PI); SC control. EN = day 8 PI; PO control. EN/NB = day 8 PI EN and day 11 PI NB; PO/SC control.

pH 7.4, with the final volume being 2.5 ml. Free radical levels were determined in the mucosa indirectly by using the TBA Assay (Asakawa and Matsushita, 1980) to measure malondialdehyde (MDA), an end product of the lipid peroxidation reaction generated by free radicals. A standard curve was prepared for use in determining MDA concentration in the mucosal homogenate samples by plotting the absorbance readings obtained from the following known concentrations of 1,1,3,3-tetramethoxypropane (malonaldehyde bis[dimethyl acetal]): 0.25, 0.5, 1, 2, and 4 nM. Absorbance was measured using a Beckman spectrophotometer at 532 nm. Protein concentrations in the homogenates were determined using the Bradford Assay, and data were expressed as x̄ nM MDA × 10^{-2}/μg protein. Data were analyzed with a multivariate analysis of variance using a Wilks' Lamda (P-value = 0.6521).

The small intestinal mucosa from all experimental groups tested positive for the presence of low levels of MDA; however, no significant ($P \geq 0.05$) differences in MDA production were found among the groups (Fig. 1).

Smith and Bryant (1989) suggested that free radicals play an important role in *N. brasiliensis* self-cure in Wistar rats. Results of the present study, however, suggest they are not important effectors of self-cure in Sprague-Dawley rats, since free radical levels during single or concurrent infection with *N. brasiliensis* and/or *E. nieschulzi* did not differ from those of uninfected control rats. These results are consistent with data obtained from mice infected with *N. brasiliensis* in our laboratory (Modric and Mayberry, 1994). The data also indicate *E. nieschulzi* does not inhibit self-cure of *N. brasiliensis* through suppression of free radical production.

Results of our TBA Assay show that low levels of MDA were produced during infection with *N. brasiliensis* or *E. nieschulzi* when compared to those levels observed by Smith and Bryant (1989) and Ovington and Smith (1992) in response to *N. brasiliensis* and *E. vermiformis,* respectively. It is possible that free radicals were produced in larger quantities in the Sprague-Dawley rats, but they were not able to participate in the lipid peroxidation reaction where MDA was formed due to the detoxification of free radicals by various host or parasite enzymes such as superoxide dismutase (SOD), catalase, and peroxidases (Ellis, 1990). In support of this argument, Batra et al. (1993) reported that *N. brasiliensis* contained SOD, glutathione peroxidase, and also catalase. It may be that the strains of *N. brasiliensis* used in our laboratory and Sprague-Dawley rats have higher levels of these enzymes than parasite or host strains used by other researchers such as Smith and Bryant (1989). An alternative explanation as to why our experiments with *N. brasiliensis* produced results that are contradictory to those reported by Smith and Bryant (1989) may be that they infected rats with 6 × 10^3 third-stage larvae of *N. brasiliensis,* while we infected with 1 × 10^3. The increased number of larvae administered in the experiments by Smith and Bryant may have resulted in an intestinal worm burden large enough to induce a significantly higher level of free radical production and, thus, MDA when compared to the MDA levels of the current study. A third explanation may be that the production of parasite-induced free radicals may vary not only with the parasite burden and strain but with the strain of host as well. These are all important factors that must be considered when extrapolating results obtained in 1 laboratory or host system to results obtained in other laboratories with different strains of host and/or parasite.

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Evolutionary Parasitology Course

The course, "Evolution of Parasites and of Host-parasite Relationships/Évolution des Parasites et des Relations Hôtes-parasites," will be held on May 11–13, 1998 in Paris, France. For further information, please contact Dr. Marie-Claude Durrette-Desset or Pr. Jean-Lou Justine, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Muséum National d'Histoire Naturelle, 61 rue Buffon, 75231 Paris cedex 05, France. Tel: +33 1 40 79 35 03; Fax: +33 1 40 79 34 99; e-mail: mcdd@mnhn.fr or justine@mnhn.fr